

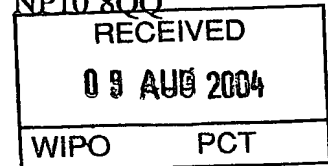


PCT/GB2004/002905.



INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ



I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Stephen Hordley

Dated 2 August 2004

**PRIORITY
DOCUMENT**

SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

Patent Act 1977
(Revised)

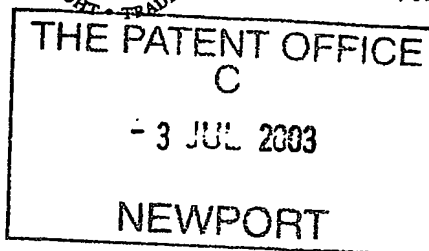


03JUL03 E819794-2 000239
P01/7700 0100-0315540.5

177

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)



The Patent Office

Cardiff Road
Newport
South Wales
NP10 8QQ

1. Your reference
PC/JD/P12627GB

2. Patent application number
(The Patent Office will fill in this part)

0315540.5

3. Full name, address and postcode of the or of each applicant (underline all surnames)

University Court of the University
of St Andrews
North Street
St Andrews
Fife KY16 9AJ

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

8250128002

4. Title of the invention
DELIVERY OF NITRIC OXIDE

5. Name of your agent (if you have one)
"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

Cruikshank & Fairweather
19 Royal Exchange Square
G1 3AE

Patents ADP number (if you know it)

547002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number	Country	Priority application number (if you know it)	Date of filing (day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application	Date of filing (day / month / year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

- a) any applicant named in part 3 is not an inventor, or
- b) there is an inventor who is not named as an applicant, or
- c) any named applicant is a corporate body.

See note (d))

Yes

Patents Form 1/77

- 9 Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form

Description 18

Claim(s)

Abstract

Drawing(s) 2 + 2

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (*Patents Form 7/77*)

Request for preliminary examination and search (*Patents Form 9/77*)

Request for substantive examination (*Patents Form 10/77*)

Any other documents
(please specify)

11. I/We request the grant of a patent on the basis of this application.

Signature

Cruikshank & Fairweather

Date

2/7/03

Cruikshank & Fairweather 2 July 2003

12. Name and daytime telephone number of person to contact in the United Kingdom

Dr Paul G Chapman

0141 221 5767

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

- If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.
- Write your answers in capital letters using black ink or you may type them.
- If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
- If you have answered 'Yes' Patents Form 7/77 will need to be filed.
- Once you have filled in the form you must remember to sign and date it.
- For details of the fee and ways to pay please contact the Patent Office.

DELIVERY OF NITRIC OXIDE

FIELD OF INVENTION

5 The present invention relates to zeolites containing releasably adsorbed nitric oxide, methods of preparing these zeolites, methods of releasing the nitric oxide into a solution or into air, and uses thereof.

BACKGROUND OF THE INVENTION

10 Nitric oxide (the chemical formula is NO) is a remarkable small molecule that is vitally important in many biological processes. It is a vasodilator that increases blood flow through arteries and veins, and is also an important factor in controlling platelet adhesion
15 and aggregation. It also plays a crucial role in the immune system. Much is now known about the mode of action of nitric oxide and it is clear that it has enormous potential in medicine and biotechnology in both in vivo and ex vivo applications.

20 The controlled delivery of nitric oxide may be important in therapy. For example, nitric oxide can prevent thrombosis and restenosis following balloon angioplasty and stent insertion in blocked arteries (International Patent Application WO 95/24908). The
25 delivery of nitric oxide to the skin may also have therapeutic benefits for patients with peripheral circulatory problems which can occur in conditions such as arthritis and Raynaud's syndrome. Nitric oxide also plays a part in wound healing and angiogenesis, and
30 delivery of nitric oxide to wounds can be beneficial when healing is slow which can occur, for example, in elderly patients (M. Shabani et al, Enhancement of wound repair with a topically applied nitric oxide-releasing polymer *Wound repair and regeneration*, 4, 353, 1996 and S. Frank

H. Kampfer, C. Wetzler, J. Pfeilschifer, Nitric oxide drives skin repair: Novel functions of an established mediator *Kidney International*, 61, 882, 2002).

5 However the delivery of nitric oxide to the desired area, and in the required optimum dose is often difficult because nitric oxide is a gas. Delivery of nitric oxide is difficult in both *ex vivo* e.g. biotechnology applications and *in vivo* e.g. medical applications.

10 Various methods of nitric oxide delivery are known such as

- (a) molecules which release NO spontaneously;
- (b) molecules which are metabolised to give NO;
- (c) molecules that release NO on photoactivation;
- (d) release of NO from polymers and polymer
15 coatings;
- (e) production of NO from a chemical reaction.

20 The class (a) molecules are known as nitric oxide nucleophile complexes (NONOates) (C.M. Maragos et al, Complexes of NO with nucleophiles as agents for the controlled biological release of nitric-oxide-vasorelaxant effects *J. Med. Chem*, 34, 3242, 1991). These are a variety of molecules which give off nitric oxide spontaneously and have been shown to have a
25 possible use in therapeutic applications (US Patent 4954526). However the use of NONOates in therapy is limited because they become distributed throughout the body which may compromise selectivity. The by-products following the release of NO may also form carcinogenic
30 secondary nitrosamines.

The class (b) molecules include glyceryl trinitrate and sodium nitroprusside (L.J. Ignarro Biosynthesis and metabolism of endothelium-derived nitric-oxide *Ann. Rev. Pharmacol. Toxicol.* 30, 535, 1990). These compounds are

currently widely used as vasodilators, however prolonged use can lead to toxic side products such as cyanides. Furthermore, because these molecules need to be metabolised to release NO, the targeting of NO to particular sites may also be poor resulting in the effects tending to be systemic.

The class (c) molecules require specific activation, for example, light having a specific wavelength which can be difficult to initiate (C. Works, C.J. Jocher, G.D. Bart, X. Bu, P.C. Ford, Photochemical Nitric Oxide Precursors *Inorg. Chem.*, 41, 3728, 2002).

Class (d) release of nitric oxide mitigates the problems associated with systemic activity by delivering nitric oxide to a specific target site by supporting a nitric oxide releasing compound on a solid article. Such NO releasing compounds may be polymeric materials which can be coated onto medical instruments which can be used to target specific areas of the body for treatment. The polymers may contain, for example, the N_2O_2 group that releases NO after a chemical reaction (International Patent Application WO 95/24908 and US Patent Application 2002094985). However, the release of NO in such circumstances can be difficult to control and currently the preparation of the required materials may be expensive. The possible use of such polymers has been shown in the treatment of cardiovascular problems, for example, restenosis.

Class (e) delivery of nitric oxide has been proposed for topical applications by releasing nitric oxide from a chemical reaction. The chemical reaction involves the application of sodium nitrite, ascorbic acid and maleic acid, which gives off NO when contacted by water (US Patent Application No. 6,103,275). However, this reaction takes place only in acidic conditions and so may

cause irritation, especially to sensitive skin of elderly patients.

The object of the present invention is to obviate and/or mitigate the problems of nitric oxide storage and delivery.

SUMMARY OF THE INVENTION

According to a first aspect of the present invention there is provided a zeolite material comprising releasably adsorbed nitric oxide.

Zeolite materials are a class of aluminosilicate materials which are known and used in a number of applications, for example, ion exchange, gas separation and catalysis (A. Dyer, An Introduction to Zeolite Molecular Sieves, J. Wiley and Sons, 1988).

According to a second aspect of the present invention there is provided a method of preparing a zeolite material which comprises releasably adsorbed nitric oxide, said method comprising the steps of providing a zeolite material and contacting said material with nitric oxide gas.

Zeolites which are suitable for the present invention may be either naturally found or synthetically made. The zeolites contain pores and channels having dimensions which allow small molecules or ions to be adsorbed onto the internal surfaces of the material. The general formula of a zeolite framework is $Al_ySi_{1-y}O_4^{y-}$. For each aluminium atom in the zeolite framework, one negative charge is introduced which must be balanced by an extra-framework cation. These cations can be inorganic or organic in nature, and can be exchanged using standard ion exchange processes (M.E. Davis, Ordered porous materials for emerging applications Nature 417, 813, 2002).

The zeolites may comprise transition element cations as the extra-framework species e.g. iron, copper, ruthenium, and such zeolites can adsorb nitric oxide to form complexes inside the cavities of the zeolite material. These complexes are strong and may enable the nitric oxide to be stored until needed. Cations of other elements, for example, sodium and potassium bind nitric oxide less strongly. Those skilled in the art may use standard ion exchange processes to introduce the required metal ions into a zeolite structure as extra-framework cations (Plank et. al., U.S. Patent No. 3,140,249; Preparation, characterisation, and Performance of Fe-ZSM-5 Catalysts R. Joyner and M. Stockenhuber, J. Phys. Chem. B., 1999, 103, 5963-5976). Using such techniques it is possible to incorporate mixtures of cations in the zeolite structures.

The zeolites may be provided in a dehydrated state.

The amount of nitric oxide which may be loaded into the zeolites can be controlled by varying the relative amounts of the extra-framework cations, controlling their chemical nature, and/or the total number of ions present. For example, the number of extra framework cations present in the zeolite structure may depend on the amount of aluminium present in the framework. More aluminium ions require more extra framework cations to balance the negative charge. The extra framework cations may then interact with the NO molecules.

The chemical nature of the extra framework cations may also be changed (for example monovalent cations, e.g. Na^+ and Ag^+ may be exchanged for divalent cations, e.g. Fe^{2+} and Cu^{2+} or trivalent cations, e.g. Ru^{3+} and Fe^{3+}). Each different cation may have a different affinity for NO and changing the cations present in the zeolite framework may be used to control the release of NO. Such

manipulation of the zeolite composition can affect the rate at which the nitric oxide is released from the zeolite. For example a sodium-loaded zeolite may bind nitric oxide less strongly than an iron-loaded zeolite to release the nitric oxide more rapidly. A mixed sodium/iron zeolite may release nitric oxide at a different rate to either a sodium-loaded zeolite or an iron-loaded zeolite, and such release of nitric oxide may present a different rate profile.

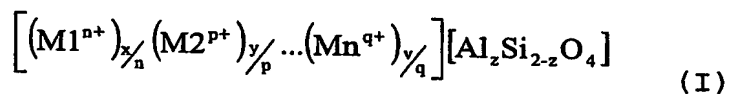
The choice of zeolite framework can also be used to vary the loading and release rate of nitric oxide. Zeolite frameworks are available in synthetic materials with a variety of different structures, and suitable frameworks may be chosen that offer the desired properties for the application under consideration. For example, the pores and channels in a zeolite structure may be defined by the size of the pore or channel openings. The zeolite with the structure LTA has openings defined by 8 pore tetrahedral units (i.e. a ring of 8Si/Al atoms and 8 oxygen atoms). Zeolite MFI has a larger ring opening defined by 10 tetrahedral units, and FAU by an even larger pore opening of 12 tetrahedral units. The dimensionality of the pores can also differ between zeolite frameworks. For example, some zeolites have channels that run in only one direction (one dimensional channel systems) while others have systems of interacting channels in two or three dimensions (2-dimensional and 3-dimensional channel systems). The size, shape and dimensionality of zeolites may affect the rates of diffusion and adsorption/desorption of NO, and may be used to control the rate of release of NO from the zeolite in a particular application.

Thus, the composition of the zeolite material may be tailored to control the amount of nitric oxide loaded

into the zeolite structure and/or the rate at which the nitric oxide is released from the zeolite.

Such zeolite structures may be chosen from, but not limited to, frameworks having the following three letter framework codes: LTA, FAU, MFI, MOR, FER, BEA, PHI and SAS (See International Zeolite Association Website www.iza-online.org for details of how the codes relate to the frame-work structures of the zeolites which is incorporated herein by reference). These three letter codes describe the framework architecture of the zeolites, that is their structure, but do not describe the composition of the zeolite which may vary widely. The three letter codes are used as a nomenclature system for zeolites.

The zeolites which may be used in the present invention may have the following general formula (I):



wherein M1 and M2 ... Mn are extra framework metal cations of elements selected from the group consisting of Li, Na, K, Ca, Mg, Fe, Cu, Ru, Rh, Co, Ni, Zn and Ag.

x may range from zero to nz,

y may range from zero to pz, and

v may range from zero to qz,

subject to the condition that $x/n + y/p + \dots + v/q = z$.

z is the number of silicon atoms replaced by aluminium atoms in the zeolite framework.

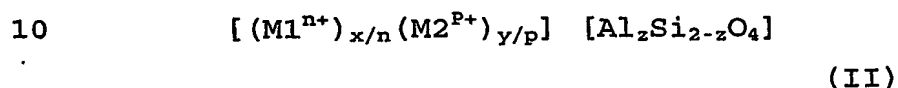
n+, p+ and q+ are the charges of the extra framework metal cations, and may individually take the values of +1, +2 or +3.

M1 and M2 ... Mn may also be chosen from small organic cations such as $N(R_1)_a(R_2)_b^+$ wherein R_1 and R_2 are

independently selected from H, -CH₃, -CH₂CH₃, or -CH₂CH₂CH₃, and a and b are independently 0, 1, 2, 3 or 4 such that a + b = 4;

When M1 and/or M2 are small organic cations, NH₄⁺ is preferred.

The zeolites which preferably may be used in the present invention have the following general formula (II):



wherein M1 and M2 are as defined previously,

x may range from zero to nz, and

y may range from zero to pz, subject to the condition that $x/n + y/p = z$.

Prior to nitric oxide loading, the zeolites for use in the present invention may be fully or partially dehydrated, for example, under vacuum to remove water from the zeolite channels. The resulting zeolite may then be exposed to nitric oxide to load the zeolite.

Typically, the nitric oxide loading is performed at a temperature from -100°C to 50°C.

The loading of nitric oxide may be performed with pure NO or with a mixture of NO and a carrier gas such as an inert gas, for example helium, argon or other inert gas including mixtures thereof.

The loading is typically performed at a pressure above atmospheric pressure, for example from atmospheric pressure up to a pressure of 10 bar.

The nitric oxide loaded zeolites may be sealed inside airtight packaging for storage and transport purposes.

Upon exposure of the nitric oxide loaded zeolite to moisture, for example an aqueous environment such as water or blood, the nitric oxide is displaced from the metal complex inside the zeolite resulting in release of nitric oxide gas into the aqueous environment.

The nitric oxide may also be released from the nitric oxide loaded zeolite when placed in air.

The release of nitric oxide may occur at a variety of temperatures, however room temperature or body temperature is preferred.

The nitric oxide loaded zeolite may be prepared in the form of a powder or a monolith for use for example in topical therapeutic applications or *in vitro* applications such as delivery of specific amounts of NO to cell cultures. For example, a specific amount of NO may be loaded into a zeolite and then, knowing the extent of release or release profile of the NO loaded zeolite, a precise amount of NO may be delivered to the cell culture. This principle may also be applied to other delivery applications of NO e.g. in therapeutic applications so that a specific amount or dose of NO may be administered.

The monoliths may be formed by compression of a zeolite powder or by mixing a powdered zeolite with a suitable binder which is well known in the manufacture of zeolite catalysts.

Suitable binders include, but are not limited to, ceramic binders e.g. silica or alumina, polymeric binders, e.g. polysulfone, polyethene, PET, polystyrene and other polymers.

Alternatively the zeolites may be provided as coatings on medical devices such as metallic medical devices. The coated devices may then be delivered to the locality where the nitric oxide is required. For

example, a zeolite coated stent may be used to perform balloon angioplasty and the release of nitric oxide under these conditions may be used to reduce restenosis.

Typically, the zeolites are provided in a suitable form as discussed above, and then loaded with nitric oxide ready for storage and use at a later time.

A powdered zeolite loaded with nitric oxide may be used in topical applications such as for wound dressing, and may be provided in a bandage for application to a wound for release of the nitric oxide into the wound to aid healing. A zeolite provided as a monolith may be used e.g. for topical applications or, for example, for suppository application in the treatment of severe constipation.

According to a third aspect of the present invention there is provided a zeolite material comprising releasably adsorbed nitric oxide for use in surgery and/or therapy.

According to a fourth aspect of the present invention there is provided a pharmaceutical preparation comprising a zeolite material comprising releasably adsorbed nitric oxide together with a pharmaceutical carrier.

The present invention also provides the use of a zeolite material comprising releasably adsorbed nitric oxide in the preparation of a medicament for use in the treatment or prophylaxis of disease. Diseases or medical conditions which may be treated include infections of the skin, including dermatophyte fungi, leishmaniasis, molluscum and papilloma virus, and mycobacterium infections. Further uses include therapeutic applications in anti-neoplastic activities, immune response modification, treatment of Raynaud's disease, wound healing and skin pigment modification. Yet further

uses include treatment of restonsis, psoriasis and eczema, and skin cancer (melanoma). Therapies for other bacterial problems include the reduction of severe foot or body odour problems, and in the treatment of
5 Methicillin Resistant Staphylococcus Aureus infections.

According to a sixth aspect of the present invention there is provided a medical article comprising a zeolite material.

The zeolite material of the medical article may be
10 provided without nitric oxide loaded therein to allow loading with nitric oxide prior to use and/or storage of the medical device ready for subsequent use.

Alternatively, the zeolite material of the medical article may be provided as a zeolite material comprising
15 releasably adsorbed nitric oxide.

Suitable medical articles for use in the present invention include a stent, catheters, wound dressings, bandages, self-adhesive plasters and patches.

The present invention also provides, as a seventh
20 aspect, a method of releasing nitric oxide comprising the steps of

- (i) providing a zeolite material comprising releasably adsorbed nitric oxide;
- (ii) contacting said zeolite material with a
25 medium into which said nitric oxide is to be released.

Such release of nitric oxide is preferably achieved in a controlled manner, for example, by providing a suitable zeolite material with an established controlled
30 release profile.

The medium into which the nitric oxide is to be released may be simply air surrounding the nitric oxide loaded zeolite, or may be, for example, an aqueous medium.

The release may be performed either inside an animal body, topically to an animal body or in non-body applications such as release into cell cultures.

5 The release may be performed at any suitable temperature, however room or body temperature is preferred.

10 The method of releasing nitric oxide may be applied to the treatment of humans or animals and accordingly the present invention further provides as an eighth aspect a method of treatment or prophylaxis of an individual in need thereof comprising providing a zeolite comprising releasably adsorbed nitric oxide and contacting said zeolite with said individual.

15 Embodiments of the present invention shall now be described with reference to the following non-limiting examples in which:

Example 1 describes the preparation of ion-exchanged zeolites;

20 Example 2 describes the preparation of nitric oxide-loaded zeolites;

Example 3 describes the release of nitric oxide from a nitric oxide loaded zeolite into the atmosphere;

Example 4 describes the release of nitric oxide from a nitric oxide loaded zeolite into solution;

25 Example 5 describes the release of nitric oxide from an alternative nitric oxide loaded zeolite into the atmosphere;

Example 6 describes the quantification of nitric oxide in solution by direct measurement.

30 Figure 1 is a graph showing the release profile of NO into the atmosphere according to Example 3.

Figure 2 is a bar chart showing the release profile of NO into the atmosphere at different times according to Example 5.

Figure 3 is a graph which shows the amount of dissolved NO concentration in accordance with Example 6.

Example 1

5 Preparation of ion-exchanged zeolites

The synthesis of zeolites is well known to those with knowledge of the art, and ion exchange of the zeolites can be carried out by standard methods (Plank et. al.,
10 U.S. Patent No. 3,140,249; Preparation, characterisation, and Performance of Fe-ZSM-5 Catalysts R. Joyner and M. Stockenhuber, J. Phys. Chem. B., 1999, 103, 5963-5976). The ion-exchanged zeolite is then dehydrated under vacuum. Analysis of the zeolites is carried out using
15 elemental analysis, X-ray diffraction and spectroscopic analysis.

An example of the preparation of a dehydrated ion-exchanged zeolite is described below.

20 The zeolite (MFI, 2g) was placed in a 0.05 M solution of the metal ion (200 ml, distilled water) to be exchanged and stirred for 24 hours. Alternatively, with the same concentrations the exchange could be carried out under
25 dry conditions in an inert atmosphere (argon) with sonication using methanol as a solvent. The products were recovered by filtration/centrifuge.

30 The concentration of the metal ion solution and time for the exchange can be varied to vary loading of the metal into the zeolite. Specific examples of different metal ions that have been loaded into the zeolites are given in Table 1.

Table 1 - Elemental composition of ion-exchanged zeolites prepared using this methodology. The table shows a range of ion exchange behaviour from very low exchange in the case of iron up to over exchange in the case of copper.

5 Initial composition of the zeolite - $(\text{NH}_4)_z[\text{Al}_z\text{Si}_{2-z}\text{O}_4]$ where $z = 0.13333$ ($\text{Si}/\text{Al} = 14$)

	Cation (M)	Final Al/M ratio
	Fe^{3+}	17.82
10	Ni^{2+}	8.42
	Co^{2+}	3.84
	Cu^{2+}	1.50

Example 2

15 Preparation of NO-loaded zeolites

Nitric oxide can be produced in situ, or introduced from a cylinder. An example of the preparation of an NO-loaded zeolite is given below.

20 A 1M asorbic acid solution (200 ml) was degassed by bubbling argon through the solution with stirring. This was then added dropwise to sodium nitrite (~5g) which had been purged with argon for 30 minutes. A slow flow of argon was used to carry the produced nitric oxide through
 25 high surface area potassium hydroxide to remove higher nitrogen oxides, and then through calcium sulfate to dry the gas stream, before being allowed to flow through the ion-exchanged zeolite (e.g., Fe-loaded MFI zeolite ~0.5g) then finally through a bubbler.

30 The NO-loaded zeolite is then sealed inside the vessel and stored under the Ar/NO atmosphere until required. The same method of NO-loading is used for all the zeolites, independent of framework type and ion exchange.

Example 35 Release of NO into the atmosphere from NO-loaded Fe-MFI
zeolite

10 Thermogravimmetric analysis coupled with mass
spectroscopic analysis of the resultant gases was used to
study the temperature dependence of the evolution of
nitric oxide from the zeolite. The results are reproduced
graphically in Figure 1 which shows the profile of weight
loss (line A) and ion current (line B) for NO in a mass
spectrometer versus temperature. NO-loaded Fe-MFI
zeolite (0.010g) was placed in a Netzch
15 Thermogravimmetric analyser coupled to a mass
spectrometer. The sample was heated to 300°C at 10°min⁻¹
48 hours under flowing air and the gases evolved analysed
using mass spectrometry. The resultant trace indicated
that the amount of NO released increases up to 130°C
20 before it begins to reduce. However, at ~180°C a sharp
spike in NO production is seen, coinciding with a phase
transition in the zeolite sample (confirmed by
differential scanning calorimetry). This is the well
known monoclinic to orthorhombic phase transition that
25 occurs in zeolite MFI. The phase transition temperature
can be altered by careful choice of the silicon to
aluminium ratio of the starting zeolite, by controlling
the ion exchanged cation and amount, and by controlling
the amount of NO loading. Thus property can then lead to
30 a tailored NO release, by for example, a heat pad applied
to a wound healing bandage - at temperatures below the
phase transition NO release is slow, while above the
phase transition NO release is much enhanced. Figure 1
shows the phase transition at 180°C, but there are

literature reports of phase transition in zeolite MFI as low as -100°C (H Morell, K Angermund, A R Lewis, DH Brouwer, C A Fyfe, H Gies Structural investigation of Silicate-I loaded with n-hexane by X-ray diffraction, Si-29 MAS NMR, and molecular modeling. Chem. Mater. 14, 2192, 2002). The precise transition temperature depends on the composition of the zeolite and the loading of NO. Other zeolites, such as FER also show phase transitions that can be tailored in this way.

Example 4

Release of NO into solution from NO-loaded Fe-MFI zeolite

Fe-MFI nitric oxide adsorbed sample (0.013 g) was placed in distilled water (10.452 ml) was tested for nitrite (Quantofix Nitrite Sticks) which give a positive result with 20 mg/l NO_2 . A sample of distilled water was tested for nitrite (as a reference) which resulted in 0 mg/l NO_2 . Nitrite is formed in solution from the reaction of NO with water and oxygen and is therefore an indirect method for the measurement of NO in solution.

Example 5

Release of NO into atmosphere from NO-loaded Fe-ZSM-5

A small sample of NO-loaded Fe-ZSM-5 (0.010g) was placed in a Netzch Thermogravimmetric analyser coupled to a mass spectrometer. The sample was heated to 37°C for 48 hours under flowing air and the gases evolved analysed using mass spectrometry. The resultant trace indicated that NO is slowly released from the zeolite at these temperatures into the atmosphere. Figure 2 shows the profile of NO

released from the zeolite at different times during the cycle. The bar chart shows ion current (from mass spectrometer) versus time for four molecules (H_2O , NO , NO_2 and HNO_2) released from NO-loaded Fe-MFI. It can be clearly seen that NO is the most abundant gas given off at all times.

Example 6

10 Quantification of NO in solution by direct measurement using a nitric oxide electrode.

The World Precision Instruments ISO-NO Mark II nitric oxide electrode was calibrated using the titration method according to the procedure described by World Precision Instruments (ISO-NO Mark II Instruction Manual, World Precision Instruments, 2002). The metal ion-exchanged zeolite with adsorbed nitric oxide was transferred into a glass tube and wet argon (5 ml min^{-1}) was allowed to flow over it. This stream was then directed to bubble through a buffered solution (pH 7.4 at 37°C) into which the nitric oxide electrode was immersed. Data on the release of nitric oxide was then collected over several hours.

25 Figure 3 shows the dissolved nitric oxide concentration (not normalised for mass of zeolite or degree of ion exchange) produced when three NO-loaded zeolite samples are exposed to a flow of moist argon as described above. The gas flow is then bubbled through the buffered solution and the nitric oxide concentration measured with time. The experiment measures the uptake of nitric oxide by the solution, and takes no account of loss of nitric oxide that does not dissolve in the liquid. However, for many of the proposed applications (e.g. as a wound-

healing bandage) where release of nitric oxide is not directly into a solution, this experiment mimics the situation more closely than would release of the nitric oxide directly into a liquid.

5 The results illustrate that different nitric oxide-loaded zeolite materials release NO in different ways. Zeolites with the LTA structure release their NO relatively quickly, while those based on the PHI framework release nitric oxide over a much longer timescale. It is noted
10 that the copper and iron ion exchanged LTA zeolites show similar release profiles. The results do show in all cases that the concentration of nitric oxide in the solution is of similar magnitude (nanomolar to micromolar concentrations) to that found in many biological
15 situations.

1/2

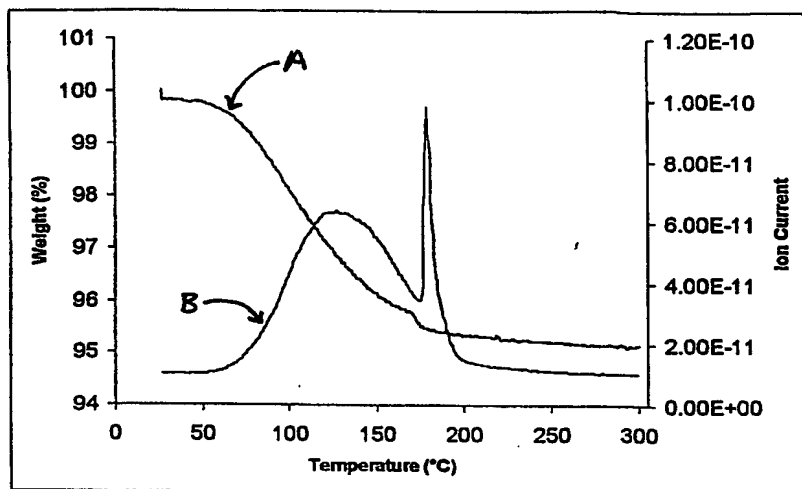


Figure 1

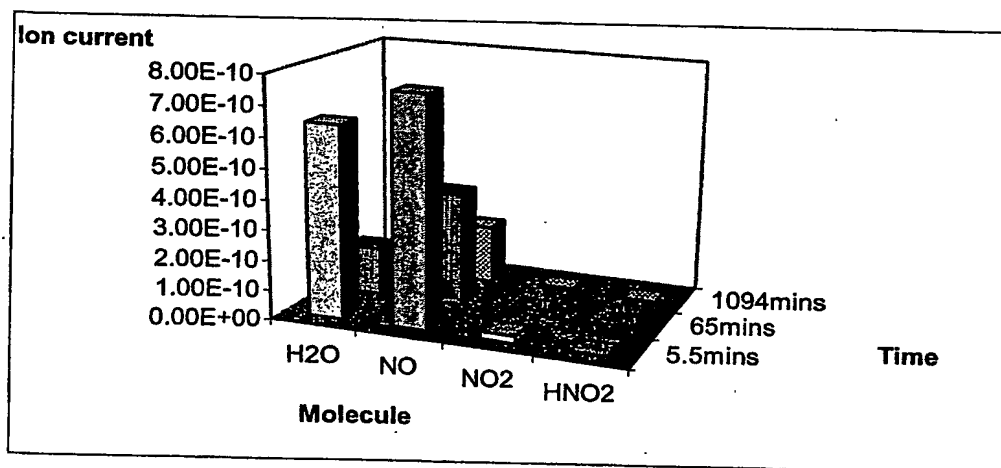


Figure 2

2/2

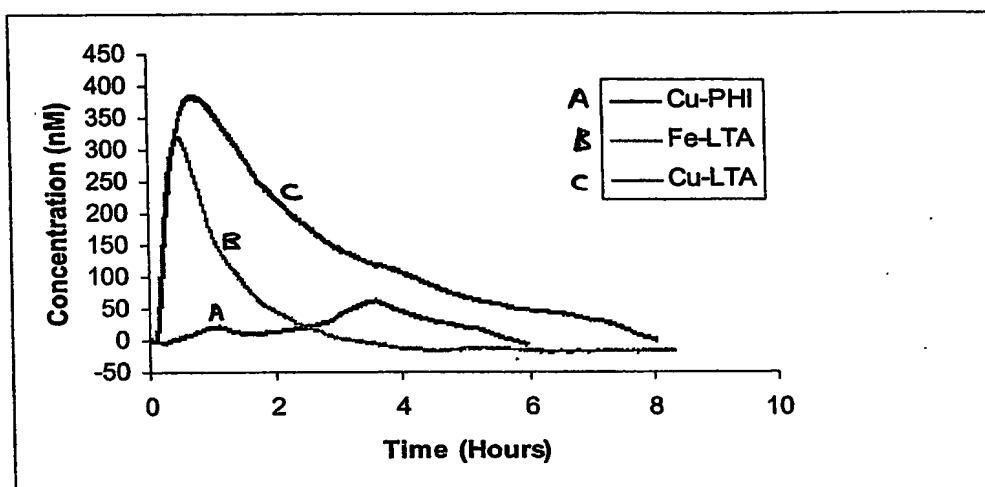


Figure 3

